

We claim:

1. Non-adsorbed insulin crystals comprising:

(a) a polypeptide selected from the group consisting of  
5 insulin, an insulin analog, and a derivatized insulin;

(b) zinc, present at about 0.3 mole to about 1 mole per  
mole of polypeptide;

(c) protamine; and

(d) a hexamer-stabilizing compound,

10 wherein less than 2% of said polypeptide is present on  
said non-adsorbed insulin crystals as adsorbed polypeptide,  
and wherein said non-adsorbed crystals have a longest  
dimension that is between 0.5 to 10 microns.

15 2. The crystals of claim 1, wherein less than 1% of  
said polypeptide is present on said non-adsorbed insulin  
crystals as adsorbed polypeptide.

20 3. The crystals of claim 1, wherein less than 0.2% of  
said polypeptide is present on said non-adsorbed insulin  
crystals as adsorbed polypeptide.

25 4. The crystals of claim 1, wherein said protamine is  
present at about 0.29 mg/ml to about 0.45 mg/ml per 0.57  
micromoles/ml to 0.64 micromoles/ml of said polypeptide.

5. The crystals of claim 1, wherein said polypeptide  
is human insulin.

30 6. The crystals of claim 1, wherein said polypeptide  
is a derivatized insulin.

7. The crystals of claim 6, wherein said derivatized insulin is an acylated insulin.

8. The crystals of claim 7, wherein said acylated insulin is B29-N $\epsilon$ -octanoyl-human insulin.

9. The crystals of claim 7, wherein said acylated insulin is B29-N $\epsilon$ -tetradecanoyl-des(B30)-human insulin.

10. The crystals of claim 1, wherein said polypeptide is an insulin analog selected from the group consisting of ArgB31,ArgB32-human insulin, and GlyA21,ArgB31,ArgB32-human insulin.

11. The crystals of any of claims 1-10, wherein said non-adsorbed crystals have a longest dimension that is between 0.5 to 5 microns.

12. The crystals of any of claims 1-10, wherein said non-adsorbed crystals have a longest dimension that is between 0.5 to 3 microns.

13. A method of preparing non-adsorbed insulin crystals, said method comprising:

(a) crystallizing ingredients comprising (i) a polypeptide selected from the group consisting of insulin, an insulin analog, and a derivatized insulin, (ii) zinc, present at about 0.3 mole to about 1 mole per mole of polypeptide, (iii) a first concentration of protamine, and (iv) a hexamer-stabilizing compound to form adsorbed insulin crystals; and

(b) combining said adsorbed insulin crystals with protamine so as to achieve a second, higher concentration of protamine to form said non-adsorbed insulin crystals, wherein less than 2% of said polypeptide is present on said non-adsorbed insulin crystals as adsorbed polypeptide, and  
5 wherein said non-adsorbed crystals have a longest dimension that is between 0.5 to 10 microns.

14. The method of claim 12, wherein less than 1% of  
10 said polypeptide is present on said non-adsorbed insulin crystals as adsorbed polypeptide.

15. The method of claim 12, wherein less than 0.2% of  
15 said polypeptide is present on said non-adsorbed insulin crystals as adsorbed polypeptide.

16. The method according to claim 13, wherein said first concentration of protamine is about 0.25 mg/ml to about 0.32 mg/ml per 0.57 micromoles/ml to 0.64  
20 micromoles/ml of said polypeptide, and said second concentration of protamine is about 10% to about 40% greater than said first concentration of protamine.

17. The method according to claim 13, wherein said  
25 polypeptide is human insulin.

18. The method according to claim 13, wherein said polypeptide is a derivatized insulin.

30 19. The method according to claim 18, wherein said derivatized insulin is an acylated insulin.

20. The method according to claim 19, wherein said acylated insulin is B29-N $\epsilon$ -octanoyl-human insulin.

21. The method of claim 20, wherein said acylated  
5 insulin is B29-N $\epsilon$ -Tetradecanoyl-des(B30)-human insulin.

22. The method according to claim 13, wherein said polypeptide is an insulin analog selected from the group consisting of ArgB31,ArgB32-human insulin,  
10 GlyA21,ArgB31,ArgB32-human insulin.

23. The method according to claim 13, wherein said ingredients further comprise a buffer selected from the group consisting of citrate, phosphate, acetate, TRIS, and  
15 glycine.

24. The method according to claim 23, wherein said buffer is citrate.

20 25. The method according to any of claims 13-24, wherein said non-adsorbed crystals have a longest dimension that is between 0.5 to 5 microns.

26. The crystals of any of claims 13-24, wherein said  
25 non-adsorbed crystals have a longest dimension that is between 0.5 to 3 microns.

27. A composition comprising the crystals of claim 1.

30 28. A pharmaceutical composition comprising the crystals of claim 1 and a pharmaceutically acceptable excipient.

29. A method of treating diabetes mellitus comprising administering the non-adsorbed insulin crystals of Claim 1 to a patient in need thereof in a quantity sufficient to  
5 regulate blood glucose levels in the patient.

30. The method of claim 29, wherein said quantity provides an insulin effect from about 8 hours to about 24 hours after administration.  
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31. The method of claim 29, wherein said quantity provides an insulin effect from about 10 hours to about 24 hours after administration.

15 32. The method of claim 29, wherein said quantity provides an insulin effect from about 12 hours to about 24 hours after administration.

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